****Note: Whenever the instrument doors are closed, WAIT for the autosampler to move and return to the position under the capillary array BEFORE clicking anything on the computer screen.**

1. Checking and Refilling Fluids

1.1. Determine if polymer needs to be added to the instrument before proceeding with instrument preparation.

1.2. If there is sufficient polymer for the run(s) and the polymer is less than one (1) week old, then ensure no bubbles are present in the lines and proceed with instrument preparation.

1.3. If the polymer is more than one (1) week old or insufficient in quantity to complete the run, then add polymer by following the **Replenish Polymer Wizard**.

1.4. Replace the respective solutions in the anode buffer reservoir (shot glass), the cathode buffer reservoir and the water reservoir daily or before each batch run.

1.5. To prepare 50ml of 1X Genetic Analyzer Buffer: Add 5.0ml of 10X Genetic Analyzer Buffer to a 50ml conical tube, then add 45ml of deionized water. Mix well.

2. Filling the Water and Cathode Buffer Reservoirs

NOTE: Wear gloves when performing the following procedure.

2.1. Close the instrument doors. WAIT for the autosampler to return to the capillary array.

2.2. Press the tray button on the outside of the instrument to bring the autosampler to the forward position.

2.3. Wait until the autosampler has stopped moving before opening the instrument doors.

2.4. Remove the reservoirs from the instrument.

2.5. Dispose of the fluids and rinse the reservoirs with warm deionized water. Perform a second rinse using deionized water on the water reservoirs and buffer on the cathode buffer reservoir.

2.6. Fill the cathode buffer reservoir to the fill line with 1X Genetic Analyzer Buffer.

2.7. Fill the water reservoirs to the fill line with deionized water.

2.8. Dry the outside of the reservoirs using a Kim-wipe. Be sure the septa strips fit snugly and flush on the tops of the reservoirs. At least once a week place a clean septa strip on each reservoir.

2.9. Place the reservoirs into the correct positions on the autosampler (the two water reservoirs in the back positions, the buffer reservoir in the front left position, and position 3 is empty).

3. Changing the Anode Buffer Reservoir (Shot Glass)

3.1. Remove the anode buffer reservoir by firmly pulling down and twisting slowly.

3.2. Discard the used buffer.
3.3. Clean and rinse the reservoir with warm deionized water then rinse with buffer.
3.4. Fill the reservoir to the fill line with 1X Genetic Analyzer Buffer.
3.5. Put the anode buffer reservoir on the instrument.

4. Preparing Samples to Run on the 3130xl

NOTE: A run corresponds to a defined set of 16 wells on a 96-well reaction plate.

4.1. Fill out the Sample Tray Worksheet with the location of ladders and samples on the 96-well tray.
4.2. Prepare the master mix for a 16-well run by combining the following in a single microcentrifuge tube:

Reagent	Volume
Hi-Di™ Formamide	Approx. 174µl
GeneScan™-500 Liz® Size Standard	7µl

4.3. Vortex the tube to mix, then spin briefly in a microcentrifuge.
4.4. In a 96-well reaction plate dispense 9µl of the formamide:size standard master mix into each well that will contain a sample. Add 9-10µl of the master mix to each blank well.
4.5. Load approximately 1.5µl of allelic ladder and 1.0µl of sample into the appropriate wells. Note: Ladder volume may be adjusted depending on lot variation.
4.6. Cover the reaction plate with the 96-well septa.
4.7. Briefly spin the reaction plate in a centrifuge to ensure the contents of each well are mixed and collected at the bottom.
4.8. Denature the reaction plate in a thermal cycler at 95^{o}C for 3 minutes.
4.9. Place the reaction plate immediately on an ice block for approximately 3 minutes or allow the thermal cycler to ramp down to 4^{o}C and let the plate remain on the thermal cycler at 4^{o}C for approximately 3 minutes.

5. Creating a Plate Record on the 3130xl

5.1. Open the 3130xl Data Collection Software.
5.2. In the task pane expand the **GA Instruments** folder.
5.3. In the task pane expand the **ga3130xl** folder.
5.4. Click **Plate Manager** in the task pane to open the plate manager window.
5.5. Click **New** to open the New Plate Dialog box.

5.5.1. Type the plate name.
5.5.2. *Optional*—Add a description for the plate.

5.5.3. Choose **GeneMapper—3130xl COMPUTER** from the Application drop down menu.
5.5.4. Choose **96-Well** from the Plate Type drop down menu.
5.5.5. Type SBI in the Owner Name box.
5.5.6. Type your initials in the Operator Name box.

5.6. Click **OK** to open the GeneMapper Plate Editor spreadsheet.

5.7. Plate Editor Spreadsheet: Complete the Plate Editor spreadsheet for the wells that will be run. For each of the columns enter the information, click the column header to select the entire column, then select **Edit> Fill down (Ctrl + D)** to apply the information to all of the selected samples.

Note: The plate records may be reused by highlighting the plate in the Plate Manager window and clicking Edit. In the Plate Editor spreadsheet select Edit> Add Sample Run (Alt + A).

5.7.1 In the Sample Name column type a name for the samples. The value 100 is automatically displayed in the Priority column.
5.7.2. In the Sample Type column choose the sample type from the drop down menu.
5.7.3. In the Size Standard column select **AdvancedGS500LIZ**.
5.7.4. In the Panel column select **Identifiler_v1**.
5.7.5. In the Analysis Method column select **AdvancedHID**.
5.7.6. In the Results Group 1 column select **Basic_Fragment_Analysis**.
5.7.7. In the Instrument Protocol 1 column select **HID_POP4_G5** for a 10 second injection or **HID_POP4_G5_22sec** for a 22 second injection.

5.8. Double check all entries and click **OK.**

6. Linking the Reaction Plate

6.1. Place the reaction tray containing the denatured DNA onto the 3130xl, positioned correctly with the notch in the lower right corner.
6.2. In the task pane expand the **3130XL** folder.
6.3. In the task pane expand the **Run Scheduler** folder.
6.4. Click **Plate View** in the task pane.
6.5. For Type of Search select **Advanced** from the drop down menu.
6.6. Enter the search criteria by choosing an option from the condition column and entering information describing the plate in the value columns. (For example: Date Last Modified—Condition: =; Value 1: 2008-01-08). Descriptive information can be entered into as few or as many categories as the user determines is necessary to narrow the search for his/her plate.
6.7. Click **Search**.

6.8. The plate records that fit the search criteria will appear in the window at the bottom of the screen. Highlight the plate record for the plate being linked by clicking on it.
6.9. Click the plate position indicator that corresponds to the plate you are linking.
6.10. Verify the plate has been linked.

6.10.1. The green run button on the toolbar is enabled, meaning the instrument is ready to run.
6.10.2. The plate position indicator for the linked plate changes from yellow to green.
6.10.3. The plate position (A or B) will appear in the Link column.

6.11. Repeat steps 6.1 through 6.10 above to link a second plate, if applicable.
6.12. Click **Run View** in the task pane to view the run schedule.

7. Starting the Run

7.1. Click the green run instrument button to begin the scheduled runs.
7.2. A dialog box stating "You are about to start processing plates…" will appear. Click **OK**.

NOTE: The green arrow will not be enabled until the plate is linked and back in the home position.

8. Monitoring the Run

8.1. Click **Instrument Status** in the task pane to monitor the status of the instrument during the run.
8.2. View the data using the Capillaries Viewer and the Cap/Array Viewer in the 3130XL folder.

IMPORTANT!!!! Always EXIT from the Capillaries Viewer and the Cap/Array Viewer. DO NOT leave these windows open for an extended period of time during a run. Leave the Instrument Status window open.

9. Reviewing the Data

9.1. To review the color data after the run has completed, click **Cap/Array Viewer** in the task pane. This shows the raw data for a selected capillary and electropherogram displays for all capillaries.
9.2. To review the sample files open the runs from the following default location: **E:\AppliedBiosystems\UDC\DataCollection\data\ga3130xl\3130XL.**
9.3. After the run is complete analyze the data by following the GeneMapper® *ID* SOP.

10. Sequence for Restarting the Instrument

10.1. Close the 3130xl Data Collection Software by clicking **Stop All** on the Service Console.
10.2. Turn off the 3130xl.
10.3. Restart the computer.
10.4. Turn on the 3130xl. Allow the instrument to cycle until the green light comes on.
10.5. Open the 3130xl Data Collection Software.

References:

Applied Biosystems 3130/3130xl Genetic Analyzers. User Bulletin. 2005 Applied Biosystems. Part Number 4363787. Rev A. (or most recent revision)

Document Revision History		
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APPROVAL SIGNATURES	Date
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